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Salt Dependent Association of Novel Mutants of TATA-Binding Proteins to DNA: Predictions from Theory and Experiments

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Abstract. The nonlinear Poisson-Boltzmann predictions of the salt-dependent association of proteins to DNA, SK_{pred}, are fairly insensitive to the choice of atomic charges, radii, interior dielectric constant and treatment of the boundary between a biomolecule and the solvent. In this study we show that the SK_{pred} is highly correlated with the conformational adaptability of the partners involved in the biomolecular binding process. This is demonstrated for the wild-type and mutant forms of the archaeon *Pyrococcus woesi* TATA-binding protein (*Pw*TBP) in complex with DNA, on which we performed molecular mechanics energy minimizations with different protocols, and molecular dynamics simulations and then computed the SK_{pred} on the resulting structures. It was found that the inter-molecular non bonded force field energy between the DNA and protein correlates linearly and significantly well with the SK_{pred}. This correlation encompasses the wild-type and mutant variants of the *Pw*TBP and provides us with a quick way to estimate the SK_{pred} from a large ensemble of structures generated with Molecular Dynamics or Monte Carlo simulations. The corresponding experimental SK_{obs} should also correlate with the inter-molecular non bonded force field energy between the protein and DNA, given that the underlying mechanisms in binding and salt-dependent effects are in fact the main contributors in the association of proteins/peptides to nucleic acids. We show that it is possible to fit experiments versus the inter-molecular non bonded force field energy between the protein and DNA, and use this relation to predict the SK_{obs} in absolute numbers. Thus, we present two novel approaches to estimate both the SK_{pred} and the SK_{obs} for *in silico* modelled *Pw*TBP novel mutants and even for TBPs from other organisms. This is a simple but powerful tool to suggest new experiments on the TBP-DNA type of macromolecular assemblies. We conclude by suggesting some mutants and a possible biological interpretation of how changes in solvent salinity affect the binding of proteins to DNA.

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1 Introduction

TATA-binding proteins (TBP) [1] are involved in the primary transcription machinery, where they recognize and bind to variant combinations of thymine (T) and adenine (A) clustered in an eight base-pair DNA stretch, which is called the TATA-element [2]. Because of the seminal role in the transcription process [3], the TBP is present in a broad range of organisms that have adapted to live in different – and some cases extreme – environments [4–6]. Yet, all of the presently known TBPs are highly homologous and similar in the folded state, as revealed from their three-dimensional crystal structures [7–9]. They have many features in common, *e.g.* two conserved Phe residues whose side-chains intercalate the DNA and cause it to kink about 80 degrees, and an overall α-β-α-β fold. However, they differ in the overall charge distribution, total net charge and hydrophobic packing, which most likely is due to the adaptation to the environment in which the organism lives. For instance, the archaeon *Pyrococcus woesi* (*Pw*) is an organism that has adapted to a life in highly saline environments [10], where it grows optimally at non-physiological elevated temperatures (around the boiling point of water) and is therefore categorized as a hyperthermophilic halophilic organism [4]. The *Pw*TBP differ substantially in charge distribution and overall net charge compared to the mesophilic human *Homo sapiens* (*Hs*) and the yeast *Saccharomyces cerevisiae* (*Sc*) TBPs. The latter two are highly positively charged ($+16e$ and $+12e$, respectively) while the *Pw*TBP has an overall net charge of zero with 25 positively and 25 negatively charged protein residues distributed over the entire protein (Fig. 1). Some of these residues are located at the DNA-binding interface or within 6 Å to any nearest DNA atom (Fig. 1). Oddly, a large portion of these interfacial residues are acidic in character, *i.e.* negatively charged. Thus, one would expect these residues having an unfavorable repulsive effect in DNA binding since they are negatively charged, as are the phosphate groups in the DNA backbone.

Recently, we reported an exhaustive comparison between the *Hs*-, *Sc*-, and the *Pw*-TBP bound to DNA using a combined Molecular Mechanics/Poisson-Boltzmann (MM/PB) computational approach (Bredenberg, Russo and Fenley – Biophysical J., in press). This was done in order to investigate salt-mediated association effects when TBP binds to DNA. In particular, we focused on the *Pw*TBP and some of its mutants that have thermodynamic experimental data reported in the literature ([11] and references therein). Our results were qualitatively in agreement with thermodynamic isothermal titration calorimetry (ITC) experiments which measure the binding constant, K_{obs}, for the formation of a complex (*i.e.* when TBP binds to DNA) at different salt concentrations. The logarithmic of the K_{obs} is then plotted against the logarithmic of the salt concentration